Advances in Brief

Induction of Immunogenicity of Monoclonal Antibodies by Conjugation with Drugs

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Abstract

Human anti-mouse antibody has been a nearly consistent result of human clinical trials utilizing murine antibodies. It is generally anticipated that the problem of human anti-mouse antibody will be reduced as genetically engineered, more human ("humanized") antibodies become available. It is not clear, however, what effect chemical modification of such "humanized" antibodies will have on their immunogenicity. The present studies utilize a mouse antibody and rat host model to explore aspects of this question. Rats injected with unmodified mouse monoclonal antibodies failed to mount anti-mouse immune responses, presumably due to their phylogenetic relatedness. In contrast, rats injected with a Vinca immunon conjugate mounted strong anti-conjugate antibody responses that were directed primarily against the linker portion of the conjugate. The in vivo serum pharmacokinetics of 125I-labeled antibody and conjugates were evaluated in rats with existing anti-conjugate antibody. The peak serum level attained was inversely correlated with the level of reactivity of the anti-conjugate antibody with the injected compound. This model provides a potentially useful tool for exploration of the immunogenicity of drug, toxin, or radionuclide monoclonal antibody conjugates.

Introduction

Monoclonal antibody-based therapies continue to be investigated both preclinically and clinically. Efforts are under way to explore the efficacy of unmodified antibodies and conjugates of antibodies with drugs, toxins, or radionuclides. To date, the majority of human clinical trials have utilized murine monoclonal antibodies with the nearly universal finding that human anti-mouse antibody is produced by the host (1-3). The field is clearly moving toward utilization of genetically engineered, more human ("humanized") antibodies with the goal of reducing human anti-mouse antibody and its negative impact on therapy (4-7). Such antibodies have undergone initial clinical evaluation with a favorable reduction in human anti-mouse antibody, suggesting that "humanization" may be able to reduce the significance of the immunogenicity issue (4, 6, 8). With regard to antibody conjugates, however, an additional problem might be predicted to arise which has not yet received significant attention. That is, chemical modification of "humanized" antibodies may render them once again more immunogenic. The present study describes a preclinical model which allows exploration of this concept. The model used mouse monoclonal antibody injected into rats under conditions in which little or no anti-mouse antibody response occurs with unmodified antibody. A drug conjugate with the mouse antibody, however, induced ACA, which was directed primarily against the linker and drug moietly of the conjugate. The effect of ACA on serum pharmacokinetics was explored.

Materials and Methods

Antibody and Conjugates

A murine monoclonal antibody directed against EGFr (9), 225 IgG1, was grown as ascites and purified using traditional protein A Sepharose affinity chromatographic techniques. The Vinca derivative DAVLBHYD was the generous gift of Mr. George Cullinan (Lilly Research Laboratories, Indianapolis, IN). DAVLBHYD was conjugated to the antibody as described utilizing a maltone linker (BAMME) (10) or a BAP-based linker (11).

Rat Immunization Protocol

Groups of young adult Harlan ASI brown rats were injected i.p. with unmodified 225 IgG1 or 225 IgG1-BAP-DAVLBHYD on a twice-weekly × 2 protocol (4 doses total). Individual animals were bled, and serum was stored frozen.

Immunoassays

Indirect Solid-Phase Antigen-binding Assay. Target antigens consisting of unmodified 225 IgG1 or 225 IgG1-BAP-DAVLBHYD were dried onto Falcon Microtest II flexible assay plates (Becton Dickinson, Oxnard, CA). Following washing, bound rat antibodies were detected using 125I-anti-rat IgG (Jackson ImmunoResearch, West Grove, PA). This reagent had no detectable cross-reactivity with mouse antibodies. The total bound radioactivity in sample wells was determined.

Fluid Phase Competition Assay. Dilutions of rat sera with demonstrated anticonjugate activity were mixed with excess quantities of 225 IgG1-DAVLBHYD conjugates, free DAVLBHYD, irrelevant conjugate, or irrelevant protein. These mixtures were added to wells of assay plates as described above, to which 225-BAP-DAVLBHYD had been attached. After incubation and washing, target-bound rat antibody was detected using the 125I-mouse anti-rat IgG.

Data are reported as averages of values from individual sera, with SE indicated.

Serum Clearance

Groups of five 225 IgG1-BAP-DAVLBHYD immunized rats were injected i.p. with 125I-225 IgG1-BAP-DAVLBHYD, 125I-225 IgG1-BAMME-DAVLBHYD, or 125I-225 IgG1. Two million cpm of labeled antibody or conjugate were mixed with 500 µg of unlabeled carrier antibody or conjugate and injected in a 0.5-ml volume. Blood samples were taken from the retroorbital plexus at the indicated time points, serum was separated, and an aliquot was evaluated for radioactivity.

Results and Discussion

Mouse monoclonal antibody (225 IgG1) was injected i.p. into rats on a twice-weekly × 2 protocol at 25 mg/kg. This protocol was chosen since it approximates an efficacious protocol with antibody drug conjugates (12). Fig. 1 shows that no detectable rat antimouse antibody responses to unmodified antibody occurred during the 45 days of the experiment. This may be due to the close phylogenetic relationship between rats and mice, such that, under this protocol, rats do not recognize mouse antibody as being sufficiently foreign to be immunogenic. It is clear, however, that under different conditions and protocols, rats are capable of responding to mouse Ig. This is evidenced...
by the positive control serum shown in Fig. 1, a rat anti-mouse serum provided by Dr. James J. Starling (Lilly Research Laboratories) that was produced in an unrelated study.

Fig. 1 also shows a parallel arm of the experiment in which 225-IgG1-BAP-DAVLBH-YD Vinca immunconjugate was injected into rats under an identical protocol. There was a very clear ACA response by day 21 that persisted throughout the remainder of the experiment. This result allows the conclusion that conjugation of DAVLBHYD to 225 IgG1 via the BAP linker caused the otherwise nonimmunogenic antibody to become significantly immunogenic under these conditions.

The next question asked was to what portion of the molecule was the rat ACA directed? To address this, a competition radioimmunoassay was run with the ACA-positive sera. The target on the plate was 225-BAP-DAVLBH-YD. As is shown on Fig. 2, there was no significant difference in the ability of diluent, bovine serum albumin, or unmodified 225 IgG1 to compete with the anticonjugate sera. This indicates that the ACA was directed to a portion of the conjugate other than antibody. In contrast, bovine serum albumin-BAP-DAVLBH-YD and 225-BAP-DAVLBH-YD both competed strongly with the anticonjugate sera, indicating that the response was directed toward the linker and/or drug portion of the conjugate. Free DAVLBHYD showed only slight competition, suggesting that the response is directed primarily toward the BAP linker. This conclusion is partially supported by the observation that a 225 IgG1 DAVLBHYD conjugate made with a different linker (BAMME) showed reduced competition relative to the conjugate made with the BAP linker.

Together, these data suggest that modification of an otherwise nonimmunogenic mouse antibody with a drug and linker renders it immunogenic, and the ACA response, in this case, was directed primarily toward the linker portion of the molecule. The reduced competition observed with a similar Vinca conjugate made with a different linker suggests that it may be possible to produce other drug conjugates using dissimilar linkers which have even less cross-reactivity. Indeed, in preliminary studies with a conjugate made utilizing a novel linker under development, we have observed no competition with the ACA generated in the present study.

We next wished to determine whether the presence of ACA would significantly alter the serum pharmacokinetics of conjugate versus unmodified antibody. Rats from the above studies, with BAP conjugate-induced ACA, were injected with 125I-225 IgG1, 125I-225 IgG1-BAMME-DAVLBH-YD, or 125I-225-IgG1-BAP-DAVLBH-YD. Fig. 3 shows that all three preparations had similar serum half-lives (35–40 h). It is notable, however, that the peak serum concentrations achieved differed, with the unmodified antibody being the highest, followed by the BAMME and BAP conjugates, respectively. This order is inversely related to the reactivity of the preexisting ACA with the free antibody, BAMME and BAP conjugates. That is, the BAP conjugate-induced ACA reacted most strongly with the BAP conjugate, which achieved the lowest peak serum level, and least strongly with the unmodified 225 IgG1, which achieved the highest peak serum level. It is tempting to speculate that BAP conjugate was rapidly cleared by the preexisting ACA. The similar half-lives suggest that the ACA may have been neutralized by the relatively high doses of antibody or conjugates that were administered.

It is interesting to note that the peak serum concentration of the BAMME conjugate was only slightly below that of the unmodified antibody and significantly higher than that achieved with the BAP conjugate. This is also consistent with the intermediate reactivity of the BAP conjugate-induced ACA with the BAMME conjugate and suggests that it may be possible to avoid a particular ACA by switching to a similar but immunologically distinct immunconjugate.
It is well understood that anti-hapten immune responses can be stimulated by conjugating small molecules (haptens) to larger carriers. Typically, highly immunogenic carriers are intentionally chosen for this purpose. It is less clear that modifying weakly immunogenic soluble proteins such as humanized monoclonal antibodies will render them more immunogenic in humans. The mouse-rat model described here may provide a tool for exploring this question preclinically. It may be possible to develop linker and chelate chemistry with low immunogenic potential. Similar studies may lead to a better understanding of the relative immunogenicities of drug, toxin, or radioconjugates and the influence of linkers on this property. With this information in hand, it may be possible to avoid the most highly immunogenic configurations or, alternatively, to sidestep developing immune responses by switching to other effective conjugates possessing minimal cross-reactivity.

References

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